IN THE SPECIFICATION

At page 1, line 24, please insert the following paragraphs:

-- More specifically, as disclosed in application WO 98/13694, published on April 2, 1998, an analysis of proteins in databases (Genbank and SwissProt) revealed that three microbes showed molecular mimicry of the bovine myelin sequence, the best one being found in 4-carboxy muconolactone-decarboxylase of *Acinetobacter calcoaceticus*, a common microbe present in soiland water supplies. Specifically, residues 110 to 118 of bovine myelin is the sequence (LSRFSWGAE) (SEQ. ID. NO: 9). In comparison, residues 41-49 of *Acinetobacter calcoaceticus* 4-carboxy-muconolactone decarboxylase (ISRFAWGEV) (SEQ. ID. NO: 8), residues 693-701 of *Agrobacter tumefaciens* beta-glucosidase (YTRFTWGAP) (SEQ. ID. NO: 10), and residues 274-282 of *Ruminococcus albus* beta-glucosidase (YTQFEISAE) (SEQ. ID. NO: 11) are all quite similar. (Alphabetic letters refer to the one-letter biochemical symbols for amino acids.)

As reported in WO 98/13694 it has been found that sera of BSE-affected cattle contain significantly high levels of antibodies to Acinetobacter species.

WO 98/13694 describes a diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals which comprises assaying antibodies present in the mammal which bind to an antigenic peptide which exhibits molecular mimicry of a mammalian myelin peptide. especially one having the sequence FSWGAEGQK (SEQ. ID. NO: 12). The term "molecular mimicry" refers to a degree of similarity (sequence homology) as between the antigenic peptide and a myelin peptide which results in the formation of antibodies which cross-react with myelin and demyelinate nervous tissue. The presence of such antibodies at elevated levels compared to those found in unaffected animals is therefore a marker for BSE which may be used to detect BSE at an early stage at which curative or other appropriate action may be taken.

The assay described in WO 98/13694 may be carried out using the whole Acinetobacter or other organism as the test antigen. Any strain of Acinetobacter having the antigenic peptide identified above may be used.

Alternatively, an isolated peptide or a synthetic form of the peptide may be used as antigen. Any suitable type of assay procedure may be used, the ELISA method being

especially convenient.

Antibody levels indicative of BSE are those which are significantly higher than the control levels. Usually, levels elevated to about two standard deviations above the controls may be taken as a positive indication but margins around this figure may be possible or desirable for purposes of caution.

WO 98/13694 includes the following illustrative Example, which is reproduced herein. The example from WO 98/13694 is based on a comparison of sera from animals known to have had BSE with sera from healthy animals.

Sera from 29 animals, which were found at post-mortem to satisfy the criteria of BSE and 18 animals which did not, were supplied by the Central Veterinary Laboratory (CVL) (New Haw, Addlestone, Surrey, UK), an executive agency of the United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF). The 18 animals which did not have BSE had been referred to CVL because of abnormal behaviour but post-mortem examinations carried out by MAFF had excluded BSE.

Furthermore, 30 sera from animals aged less than 30 months (A < 30M) (8 Friesians, 21 Hereford-Friesian and 1 Charolais-Friesian crossbreeds) and 28 sera from animals aged more than 30 months (A > 30M) (all dairy Friesians), were used as further controls. These were collected from a farm, kept under organic farming conditions where no case of BSE had been reported. Serum samples were obtained during routine herd testing.

Acinetobacter calcoaceticus was obtained from the National Collection of Industrial and Marine Bacteria Ltd. NCIMB 10694 (Aberdeen, Scottland, UK). Cultures were grown in 21 flasks on an orbital shaker for 2 days at 30°C, in 200 ml nutrient broth (Oxoid; 25 g/l). Flasks were inoculated with 10 ml of the corresponding starter culture left shaking at 37°C for 6 hours. Batch culture cells were harvested by centrifugation at 6000 rpm. for 20 minutes at 4°C (MSE 18, 6 x 250 ml rotor). The pellets of cells were then washed three times with 0.15 M phosphate-buffered saline (PBS: pH 7.4) before being finally resuspended in 20 ml of PBS. A stock solution of the suspension was prepared by diluting in 0.05 M carbonate buffer (pH 9.6) to give an optical density (OD) reading of 0.25 on the spectrophotometer (Corning Model 258).

The ELISA assays reported in WO 98/13694 were carried out in the conventional

manner. Briefly ELISA plates were coated with bacteria overnight at 4°C and the non-specific sites blocked with PBS containing 0.1% Tween, 0.2% ovalbumin (Sigma, Grade III), plates washed and a 1/200 dilution of test or control serum added. The plates were incubated at 37°C for I hour. washed and rabbit anti-cow immunoglobulin (IgG + IgA + IgM) (1:4000) (DakoLid.) added. The plates were re-incubated for 2 hours, washed and substrate added. The reaction was stopped with a 2 mg/ml solution of sodium fluoride (Sigma). The plates were read at 630 nm on a microtiter plate reader (Dynatech MR 600) and results expressed as OD ± S.E. All studies were carried out under code in that the tester did not know which were test or control sera. The mean OD units of total immunoglobulin antibodies in different groups were compared using Student's t-test.

Each ELISA run reported in WO 98/13694 was carried out using the following protocol:

1. Dilute antigen in coating buffer, add 200 µl to each well. Incubate overnight at 4°C wrapped in foil.

2. Wash out the antigen using washing/incubation buffer; the wells of the tray should be completely full during the washing stages as the Tween-20 prevents any further protein from being absorbed onto the plastic. Wash 3 times, leaving for approx. 4 minute intervals at room temperature.

3. Incubate the plate at 37°C for 1 hr with 0.2% Ovalbumin in washing/incubation buffer.

4. Add 200 µl of test serum. Dilutions are made in washing/incubation buffer. Incubate for 2 hours at 37°C wrapped in foil.

5. Repeat washing process as in 2.

6. Add 200 µl Horseradish peroxide HRP-conjugated second antibody diluted in washing/incubation buffer.

7. Repeat washing process as in 2.

8. Add 200 µ1 substrate (ABTS) to wells; leave to develop color for approx. 20 minutes in the dark at room temperature. Stop reaction with 100 µ1 of stopping solution and read plate at 630nm.

The results found in WO 98/13694 were as follows: Antibodies to *A.*

calcoaceticus of total immunoglobulin (IgG + IgA + IgM) were significantly elevated in the BSE sera (mean ± SE: 0.99 ± 0.05) when compared to CVL controls (0.65 ± 0.06) ($t = 4.48$, $p < 0.001$), organic farming controls aged more than 30 months (0.57 ± 0.03) ($t = 7.19$, $p < 0.001$) and organic farming controls aged less than 30 months(0.53 ± 0.02) ($t = 8.64$, $p < 0.001$).

The conclusion drawn from WO 98/13694 is that in at least in one form of "transmissible spongifonu encephalopathy" (TSE), namely BSE, a specific immune response can be demonstrated against a microbe that is found readily in the environment of cattle and which also happens to possess a molecular sequence resembling bovine myelin.

According to the present invention, a method fordetecting ade-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an Acinetobacter species. We believe that infective microorganisms of these species present to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal. The phenomenon of molecular mimicry has been explained in our above-mentioned prior application WO 98/13694, the contents of which are hereby incorporated by reference.

Similarly, the inventor's prior application WO 99/47932, published on September 23, 1999 and mentioned above, describes the presence of elevated levels of certain antibodies in human sera of patients suffering from multiple sclerosis (MS). These are the IgA antibodies to Acinetobacter species *e.g. Acinetobacter calcoaceticus*, the same organisms for which antibodies were previously found in BSE sera.

WO 99/47932 also reports that similar results have been obtained for Creutzfeldt-Jakob disease (CJD). Tests for antibodies in sera from patients who had died of CJD also show increased levels, this being especially marked for the IgA antibody sub-class. The same IgA specificity also applies to bovine sera used for the tests described in WO 98/13694.

WO 99/47932 concludes that it is clear that humans suffering from MS and CJD and cows suffering from BSE all have very significantly raised levels of *Acinetobacter calcoaceticus* IgA antibodies in their blood. Tests for such antibodies in sera from living subjects at an early stage make it possible to identify those liable to develop these

diseases. According to WO 99/47932, this opens up the opportunitv of early treatment of these infections *e.g.* by use of an appropriate antibiotic to prevent further autoimmune attack on the subjects' own myelin.

As also indicated in WO 98/13694, *Acinetobacter calcoaceticus* is one species of Acinetobacter which provides an antigen which stimulates the formation of antibodies which cross-react with the mammalian myelin.

Antibodies have been demonstrated to react with several strains of this species including 17905, AC606, SP13TV, 105/85, and11171. These strains are in the Reference Centre for Acinetobacter species held by Dr Kevin Towner, Public Health Laboratory, University of Nottingham, United Kingdom.

In carrying out the invention described in WO 99/47932, the test is for antibodies which bind to an epitope present in or derived from the Acinetobacter species. The antigen used in the test may be the whole organism or at least one prepared peptide sequence corresponding to an Acinetobacter epitope. Alternatively, peptide sequences may be used which have minor variations in amino acid sequence from the above-mentioned epitopes or prepared peptides but are conformationallv sufficiently similar to them that they also bind to the relevant antibodies. For example, peptides having the sequence RFSAWGAE (SEQ. ID. NO: 13) or ISRFAWGEV (SEQ. ID. NO: 8) may be used.

As noted in WO 99/47932, a test kit for implementing the invention therefore contains at least one test antigen as just indicated. In order to reveal IgA antibodies, the kit also contains a secondary antibody against the human, bovine, or other mammalian IgA. As indicated in WO 98/13694, antibodies are assayed and a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with Acinetobacter is via the mucous membranes of the body.

The ELISA protocol disclosed in WO 99/47932 is the same as that disclosed in WO 98/13694. The results from WO 99/47932 show that human patients suffering from

MS and CJD are statistically more likely (at a 95% confidence limit) to have elevated levels of IgA antibodies specifically reactive against Acinetobacter. --